Attorney Docket No. P58126US1

BEFORE THE BOARD OF PATENT APPEALS AND INTERFERENCES
IN THE UNITED STATES PATENT AND TRADEMARK OFFICE

In re application of Metin COLPAN

Application No. 08/796,040 Group Art Unit 1623

Filed February 5, 1997 Examiner L. Crane

For DEVICE AND A PROCESS FOR THE ISOLATION OF NUCLEIC ACID

APPEAL BRIEF

Mail Stop Appeal Brief – Patents
Commissioner for Patents
P.O. Box 1450
Alexandria, VA 22313-1450

The present brief on appeal is submitted in triplicate, subsequent to the Notice of Appeal filed January 10, 2005.

(1) REAL PARTY IN INTEREST

The real parties in interest is Qiagen GmbH, a corporation of Germany, pursuant to assignments recorded in the United States Patent and Trademark Office (PTO) on August 2, 1994, at reel/frame 7121/0455.

(2) RELATED APPEALS AND INTERFERENCES

There are no other appeals or interferences.

(3) STATUS OF CLAIMS

Claims 1-119 were canceled. Claims 120-138 (found in the Appendix) are pending and finally rejected under 35 USC 103(a) as being allegedly unpatentable over US 5,057,426 (Henco), in view of US 5,075,430 (Little) and, further, in view of *International Dictionary of Medicine and*

Biology, 1, 1986, page 522 (International Dictionary), and *Nucleic Acid Hybridisation - A Practical Approach*, 1985 pages 64, 65, and 235 (Hames).

(4) STATUS OF AMENDMENTS

There are no amendments after final rejection. An information disclosure statement was filed after final rejection, i.e., on March 10, 2005. Applicants have received no communication from the PTO concerning the information disclosure statement.

(5) SUMMARY OF INVENTION

The presently claimed invention provides a process for isolating and purifying nucleic acids found in cells, i.e., using the cells as the starting materials (application page 1, lines 1-3). The process involves two chromatographic purification (adsorbing-desorbing) stages, operated in tandem; whereby, purified nucleic-acid-containing material obtained in the first stage is applied to the second stage, directly, as it comes from the first stage.

In the first stage cells containing the nucleic acids to be isolated are digested, cell debris is removed, and the nucleic acids are adsorbed on anion-exchange material (application page 5, lines 7-36) and, subsequently, desorbed from the anion-exchange material; adsorption of the nucleic acids being effected in the presence of a buffer solution (Abstract, lines 7-8) under conditions of lower salt concentration than the buffer solution enabling the nucleic acids to be desorbed from the anion-exchange material (application page 6, lines 7-11).

In the second stage the nucleic-acids desorbed from the anion-exchange material, still in the higher-salt desorption buffer, are applied and adsorbed onto a mineral support (application page 8,

lines 1-4), followed by desorbing the nucleic acids from the mineral support using a buffer solution having a lower ionic strength than the buffer in which the nucleic acids were applied (to the mineral support) (Abstract, lines 17-19).

The combination of adjusting the ionic strength of adsorption and dessorption conditions with the adsorbing materials used in the two stages (i.e., anion-exchange material and mineral support) in accordance with the presently claimed process, allows the *desorbing* buffer in the first stage to act as the *adsorbing* buffer in the second stage. Preferably, the anion-exchange material is based on a support matrix (porous or non-porous) of surface-modified agarose, dextran, cellulose, acrylic amide, poly(vinyl alcohol), polystyrene, glass, aluminum oxide, titanium dioxide, zirconium dioxide, or, silica gel (application page 5, lines 17-21 and 24-28; page 12, lines 19-24; original claim 13). The anion-exchange material is preferably comprised of a porous or non-porous material (application page 5, lines 24-28; original claim 14) having a particle size of 1-250 μm (application page 5, lines 32-33; original claim 14); more preferably the particle size is 10-30 μm (original claim 14).

Preferred support materials are silica gel, glass, zeolite, aluminum oxide, titanium dioxide, zirconium dioxide, kaolin, diatomacae, or silica glass (application page 12, lines 29-32).

Preferably, each of the stages includes a "conditioning" step (i.e., between the adsorbing and desorbing steps), in order to optimize yields (application page 6, lines 23-26). A particularly preferred conditioning solution used in the second stage (i.e., applied to the mineral support between adsorbing and desorbing steps) corresponds to an ionic strength of about 1.5 M sodium perchlorate at a pH of approximately 5 (application page 6, lines 30-33).

The presently claimed process provides unique advantages for the purification of nucleic acids in the recited tandem *order* of using the "anion-exchange material" and "mineral support," that is, the exchange material, first, followed by the mineral support. Certain impurities, while being adsorbed to, and eluted from, the anion-exchange material along with the nucleic acids are incapable of adsorbing to the downstream mineral support under the same ionic conditions under which eluting from the anion-exchange material occurred. In other words, the buffer of higher ionic strength used to elute nucleic acids from the anion-exchange material not only allows for adsorption of the eluted nucleic acids to the mineral support; it does not provide conditions for adsorbing the impurities to the mineral support (application page 9, lines 3-10).

(6) ISSUES

The issue presented on appeal is whether claims 120-138 would have been obvious under 35 USC 103 based on the combined teachings of Henco, Little, International Dictionary, and Hames.

(7) GROUPING OF CLAIMS

The claims do not stand or fall together. Each of claims 122-126 and 129-136 is independently patentable.

(8) ARGUMENT

Claims 120-138 were finally rejected under 35 USC 103(a) based on the combined teachings of Henco, Little, International Dictionary, and Hames. The rejection cannot be sustained.

The rejection is fatally defective because the cited references, taking the teachings of each reference as a whole, neither describe nor suggest a process that contains process step "c)," as recited

in the present claims. That is, starting with the nucleic-acid containing buffer-solution product of anion exchange separation/purification of stage 1, the process step of

> c) adsorbing the separation/purified nucleic acids in the second buffer solution onto the surface of a mineral support material

(claim 120). In other words, the cited prior art does teach or suggest obtaining a solution of "twice purified nucleic acids" in a 2-stage process of (stage i) anion exchange extraction followed by (stage ii) further purifying the once-purified nucleic acids by inorganic solid phase extraction.

As broadly claimed (in claim 120) the instant invention is a process whereby "twice purified nucleic acids" are obtained from cells through the use of *two* separation/purification *stages* – stages "i)" and "ii)" recited in claim 20 – each stage involving two process steps.

In stage i): Cells are digested, the cell debris removed, and the nucleic-acid containing residue applied to anion exchanger material, under conditions that selectively bind the nucleic acids to the anion exchanger – freeing the nucleic acids from, *i.a.*, cell proteins. The protein-free nucleic acids are, then, washed out of the anion exchanger with a buffer solution "effecting [once] purified nucleic acids in the . . . buffer solution" (claim 20) – the product of stage i).

In stage ii): The one-purified nucleic acids in the buffer solution, from stage i), are applied to a mineral support, under conditions that specifically bind the nucleic acids to the mineral support. The nucleic acids are, then, washed from the mineral support "effecting twice purified nucleic acids" (claim 20).

Reliance on Henco and Little to meet the present claims is misplaced.

The process according to the instant claims saliently differs from Henco in that steps c) and d) of the present claims are neither taught nor suggested. Henco contains no motivation to modify the process disclosed in therein by the steps c) and d) of the present claims. No hint is given in Henco that (i) an increase in salt concentration should be effected in the sample fraction, nor is there any hint that (ii) such a fraction should be subsequently treated by application to a mineral support material in order to bind thereto the nucleic acid contained in the fraction, nor is there any hint to (iii) subsequently elute the substrate-bound nucleic acids using a buffer having very low ionic strength.

Little provides no teaching or suggestion to supply the salient deficiencies in Henco. Almost the same distinction with Henco applies with regard to the distinguishing Little from the presently claimed process. Applicant could not find any passage in the whole disclosure of Little that nucleic acids, ***which have already been separated,*** should be subjected to a treatment according to the process of Little. Therefore, there is indeed no motivation to combine the two documents, either in modifying Little according to Henco or in modifying Henco according to Little; or that any motivation is provided in the art to look to Henco or Little as suggested by the statement of rejection.

Applicant respectfully submits that the combination of Henco and Little is overly simplistic. Again, Henco discloses purification of nucleic acids by an anion exchange treatment or an anion exchange separation process. The key features are binding the nucleic acid at low ionic strength and eluting the nucleic acids at concentrations in the range of 2 M salt in the buffer (the number can be derived from Fig. 4 of the specification of Henco). No use of any material for being a chaotropic salt is disclosed or suggested in Henco.

According to the statement of rejection, the "choice of a specific chaotropic agent to be included in an elution buffer is a variation in chromatographic procedure which statement of rejection asserts is clearly within the perview [*sic*] of the ordinary practitioner unless applicant has shown unexpected results" (Examiner's answer, page 13). The alleged "variation in chromatographic procedure" being within the knowledge "of the ordinary practitioner" merely points out that the skilled artisan would have known *how* to vary the chromatographic procedure taught in the prior art had the skilled artisan thought up the *idea* of doing so, in the first place. With all due respect, the statement of rejection's argument fails to take into account that *invention* comprises both the *idea* of the invention and the *means* to achieve that idea. *In re Cocer*, 175 USPQ 26 (CCPA 1972). Both the idea and means to achieve the idea must be evidenced in the prior art in order to demonstrate lack of patentability. *Id.* That a difference with the prior art amounts to an alleged "optimal condition" is "not a substitute for some teaching or suggestion supporting an obviousness rejection." *In re Rijckaert*, 28 USPQ 2d 1955, 1957 (Fed. Cir. 1993). Again, both the *idea* and the *means* to achieve the idea must be evidenced in the prior art in order to show obviousness. *In re Hoffman*, 37 USPQ 222 (CCPA 1938). "That which is within the capabilities of one skilled in the art is not synonymous with obviousness [citations omitted]. *Ex parte Levengood*, 28 USPQ 2d 1300, 1302 (Bd. Pat. App. & Inter. 1993). Whether or not Little accidently uses a substance that happens to be, assuming arguendo, a chaotropic agent, but does not use it for that purpose, does not suggest use of the material as a chaotropic agent to one of ordinary skill in the art. *Minnesota Mining & Manufacturing Co., supra.*

On the other hand, Little binds nucleic acids from a solution having a very high content of salts, especially chaotropic salts.

The skilled artisan would not have had any incentive to even increase the "high" salt concentration obtained after Henco's process after reading Little's disclosure. That optional desalting is taught in Henco by the procedures disclosed therein is not disputed, but it fails to support the allegation that the skilled artisan would have been motivated to rely on Little's process in order to "desalt" Henco's sample. According to Henco, if desired, the skilled artisan would, regardless of the circumstance, try to reduce the salt content; either by applying a salt concentration, as low as possible, in the eluting step or by trying to desalt the sample by well known conservative methods, such as dialysis or gel permeation chromatography.

By no means however, would the skilled artisan ever consider, as opposed to getting rid of the salt, actually *increasing* after elution the salt content of the sample in Henco's process in order to obtain a sample having a very, very high salt concentration, as required in Little. The fact that, in accordance with the presently claimed invention, there is performed the step of increasing the salt content after Henco's process, in order to be able to employ process steps as disclosed in Little, may be regarded as a key unexpected step of steps as disclosed in the present invention.

The statement of rejection maintains that Little contains motivation to substitute the three desalting methods used in Henco (column 7, lines 44 to 46) with the silica separation method according to Little. The statement of rejection is mistaken.

Henco **starts** with DNA having a relatively low concentration of salt, which is not comparable with the situation Little addresses in the introductory portion of his disclosure. The DNA fractions dealt with in the paragraph cited by the statement of rejection are obtained after a cesium chloride gradient centrifugation. With respect to the samples which would be obtained in "too high a dilution," applicant submits that Henco teaches a method for separating DNA, wherein the DNA is not highly diluted in the eluate obtained from the method. Since the DNA is first absorbed on the chromatographic matrix and is afterwards desorbed in one elution step, the concentration of DNA is considerably high in the feral eluate. By analogy, therefore, Little's separation would be considered by the skilled person as an alternative *separation* method for isolating DNA; not as a mere substitute desalting step.

On the other hand, the method of the presently claimed invention utilizes, for the first time, the *effect* of silica disclosed in Little for such desalting steps. Originally, Little was not at all dealing with a desalting method, but with a separation method starting with highly concentrated salt solutions. This is evident from column 2, line 17 et seq. of Little, were it is stated: "This invention is directed to a process for the *purification* of plasmid and other DNA, both single-stranded and double-stranded, by immobilizing the DNA onto diatomaceous earth particles and eluting the DNA with water or low salt buffer (emphasis added)."

Therefore, the skilled artisan would not have considered using the procedure of Little as the optional desalting step of Henco. The argument made in the statement of rejection is a matter of hindsight; picking out features of the claimed process and trying to find the features in some piece

of prior art. "One cannot use hindsight reconstruction to pick and choose among isolated disclosures in the prior art to deprecate the claimed invention." *In re Fine*, 5 USPQ2d 1596, 1600 (Fed. Cir. 1988). Little is not concerned in any way with desalting of a sample. Little is concerned with purification of DNA found in a high-salt solution. Henco, however, does not yield such a sample having nucleic acid in a high salt environment.

The statement of rejection takes out of context certain statements made in Little; which distorts what is, actually, described by the reference. That is, Little states "the invention is directed generally to the immobilization of DNA onto diatomaceous earth which comprises contacting the DNA with the diatomaceous earth in the presence of a chaotropic agent" (Little column 2, lines 32-35). Similar statements are made, elsewhere, in the reference. The statement of rejection characterizes these references in a manner that makes it *appear* as if Little encompasses (that is, *contemplated*) using *isolated* DNA as a *starting material.*

On the contrary, Little was concerned with the *desire* "to rapidly and inexpensively *separate* and purify DNA that was also amenable to scale-up" (Little column 1, lines 66-67). Little contemplated purifying DNA from "bacterial lysates" (Little column 1, lines 11-12); "plasmid DNA *from mini-prep lysates* can be purified using the process of the present invention" (Little column 5, lines 43-44), "this example illustrates that DNA can be purified *from bacterial lysates* independently of the method used to prepare the DNA and *without prior phenol extraction to remove proteins*" (Little, example 1) "the isolation of supercoiled DNA *from an agarose gel* by binding onto diatomaceous earth" (Little example 4), "nucleoside from triphosphates *are effectively removed from*

radiolabeling reactions by the process of the present invention" (Little example 5), "the removal of linkers *from cloning reactions* using the process of the present invention" (Little example 6). Accordingly, Little contemplated, and described, a process that would address the problem whereby the "purification of plasmid DNA from bacterial lysates is a rate-limiting and time-consuming step in molecular biology" (Little column 1, lines 11-13), and fulfilled the objective whereby "a method was still desired *to rapidly and inexpensively* separate and purify DNA that was also amenable to scale-up" (Little column 1, lines 66-68).

As a result, the desirability (that is, motivation) provided by Little was to develop a process for isolated and purifying DNA that was more *rapid* than known methods. This motivation would not have led one of ordinary skill in the art to combine Little with Henco since it would not have sped up the process of either Little or Henco, at all; in fact, it would have increased the time over and above that needed to perform either the Henco process or the Little process. If there were any motivation, it would have been to *replace* the Henco method, entirely, with the Little method; which, also, would have effected the optional *desalting* step taught by Henco.

The rejection uses impermissible *hindsight*; that is, by selectively picking and choosing from Little's teachings in a manner that fails to appreciate Little, as a whole.

> It is impermissible within the framework of §103 to pick and choose from any one reference only so much of it as will support a given position, to the exclusion of other parts necessary to the full appreciate of what such reference fairly suggests to one of ordinary skill in the art.

In re Hedges, 228 USPQ 685, 687 (Fed. Cir. 1986).

Furthermore, the suggested combination would destroy the invention upon which Little was based; that is, for example, a *one*-step procedure to save time. A reference cannot be used (under § 103) to show obviousness in a manner that destroys the invention on which the reference is based. *In re Gordon*, 221 USPQ 1125 (Fed. Cir. 1984).

The rejection, also, is fatally defective because reliance on *International Dictionary* and Hames is misplaced.

International Dictionary and Hames are relied upon to allegedly show that Henco describes the use of "chaotropic" salts and, therefore, is allegedly appropriately combined with the teachings of Little (cited in the statement of rejection) which teaches the binding of nucleic acids found in a solution having a high concentration of "chaotropic" salts. . . .

International Dictionary and Hames add nothing to cure the fatal deficiencies found in the Henco and Little references . . . . The statement of rejection relies on the two newly cited references to allegedly show that materials disclosed in the previously cited prior art fall within the definition of "chaotropic agents." However, even assuming, arguendo, the statement of rejection is correct, the statement of rejection acknowledges that the "Henco reference does not make specific reference to a chaotropic agent." Whether or not, however, materials described by Henco accidentally fall within a broad definition of "chaotropic agent," Henco neither teaches nor suggests use of these materials for their allegedly *chaotropic* function. Therefore, even should the newly cited references show that materials described in the originally cited prior art fall within the definition of chaotropic agent, there remains no teaching or suggestion in the prior art for using these materials as chaotropic agents; and,

the statement of rejection's argument that they might function as such provides no reason or motivation for one of ordinary skill in the art to combine the materials in the manner presently claimed. Claims do not read on the prior art if "chemicals, although present in the prior art, were used for other non- . . . [claimed] functions and did not [perform the claimed function] . . . as . . . understood from the . . . specification." *Minnesota Mining & Manufacturing Co. v. Johnson & Johnson Orthopaedics, Inc.*, 24 USPQ 2d 1321, 1327 (Fed. Cir. 1992).

According to the statement of rejection, Applicant's arguments are not persuasive for failing "to provide a clear statement of how the combination of Henco et al. and Little has in any way destroyed the invention of Little as delineated by the claims found at the end of Little." The statement of rejection's reliance on the "claims found at the end of Little" as to what Little taught to one of ordinary skill in the art is misplaced. It is well-established that a patent's *claims* are no measure of what a patent discloses for prior art purposes of 35 U.S.C. 102 and 103. *In re Benno*, 226 USPQ 683 (Fed. Cir. 1985). Indeed, it is the teachings of Little, as a whole, not merely those of Little's claims that must be applied to the presently claimed invention for purposes of analysis under §103 of the statute.

The statement of rejection mischaracterizes Applicant's arguments as based on destroying "the motivation provided by Henco." Applicant made no argument about destroying "the motivation provided by Henco"; it is the motivation alleged by the statement of rejection to be found in the prior art, which Applicant disputes. It is the combined teachings of the prior art, taken as a whole, which must be considered in an obviousness analysis. *Ryko Manufacturing Co. v. Nu-Star, Inc.*, 21 USPQ

2d 1053 (Fed. Cir. 1991). Therefore, increasing the salt concentration in accordance with the teachings of Little is *not* "irrelevant to the question of motivation" to combine the prior art, contrary to the statement of rejection's argument.

According to present claim 125 a further washing step can be introduced between step c) and d) by applying an aqueous alcoholic solution. According to claim 136, which is dependent from claim 125, the alcoholic solution may include 1 to 7 M sodium perchlorate, 1 to 7 M guanidine hydrochloride, 1 to 6 sodium iodide or 1 M sodium iodide or 1 M sodium chloride in 20 % ethanol, propanol, iso-propanol, butanol, poly(ethylene glycol) or mixtures thereof.

It is no doubt true that the salts sodium perchlorate, guanidine hydrochloride, and sodium iodide belong to the group of chaotropic substances or chaotropes according to the so-called Hofmeister series. The counterpart of these chaotropes are the so-called kosmotropes.

The terms 'chaotrope' (disoder-maker) and 'kosmotrope' (order-maker) originally denoted solutes that stabilized, or destabilized respectively, proteins and membranes. Later they referred to the apparently correlating property of increasing, or decreasing respectively, the structuring of water.

Large singly charged ions, with low charge density (e.g. $H_2PO_4^-$, HSO_4^-, HCO_3^-, I^-, Cl^-, NO_3^-, NH_4^+, Cs^+, K^+ and tetramethylammonium ions; exhibiting weaker interactions with water than water with itself), are chaotropes whereas small or multiply-charged ions, with high charge density, are kosmotropes (e.g. SO_4^{2-}, HPO_4^{2-}, Mg^{2+}, Ca^{2+}, Li^+, Na^+, H^+, OH^- and HPO_4^{2-}, exhibiting stronger interactions with water molecules than water with itself).

From the above it clearly can be seen that sodium chloride cannot be classified as the "classic" chaotrope substance - on the contrary sodium chloride seems more to be qualified as a kosmotrope in the sense of the Hofmeister series.

Having in particular regard to this situation applicant takes liberty to refer to the fact that the examiner takes in the Office communication dated October 26, 2001 the point of view, that "sodium chloride clearly should be a chaotrope" [page 10, lines 19 to 25]:

> The Henco reference does not make specific reference to a chaotropic agent. In addition, the elution buffers used in Henco contain various proportions of NaCl, a compound notorious well-known in the art to alter the structure of water, and therefore NaCl must be also a chaotropic agent.

This is a fatal misinterpretation in the light of the Hofmeister series.

However, the question raised by the Examiner in the Office communication (dated September 17, 2002) focuses on the use of urea as a chaotropic compound. The Examiner relies on two additional references. From appellants' point of view it cannot be seen that these two references would be of any help with regard to the proceedings or how these references support the Examiner's position.

Henco is concerned with a method for the separation of long-chain nucleic acids from other substances in solutions containing nucleic acids and other materials, comprising fixing long-chain nucleic acids in a nucleic acid-containing solution in a porous matrix, washing the porous matrix to separate the other substances from the long-chain nucleic acids, and removing the fixed long-chain nucleic acids from the porous matrix.

The object of Henco [column 4, lines 38 to 61] (*emphasis added*) is to provide

a process for removing long-chain nucleic acids from tissues and body liquids which:

a) in a similar manner allows the nucleic acids to be extracted and concentrated from various starting materials, such as tissue, blood, sputum, cell cultures, bacteria, fungi, renal and fecal excrements, as well as from vegetable tissue from callus cultures, roots, etc.;
b) requires no long-time centrifugation steps, and more specifically no ultracentrifugation;
c) can be carried out without expensive equipment, and more specifically without refrigerated centrifuges and ultracentrifuges, and without using valuable material, such as caesium chloride for density gradients or rotor insertions for one-time use;
d) ensures high purity of the nucleic acid to be attained.
e) works without a phenolic extraction step; and
f) is suitable for being automated;

and by means of extraction of the long-chain nucleic acid, separates mixtures of long-chain nucleic acids and other materials, such as those obtained when products are biotechnologically produced.

Clearly, Henco refers to the use of urea at different places of the disclosure, mostly in cases where the biological samples for the long chain nucleic acid isolation are bacteria or viruses. For example:

Column 8, lines 59 to 63:

> The method according to the invention utilizes the described porous matrix by lysing the CMV viruses in situ by addition of urea, detergent and buffer, whereupon the DNA (130 to 150 × 10^6 Dalton) is released.

Column 12, lines 33 to 38 [Example 2]:

Upon simultaneous addition of 4 M of urea, the DNA of the phages is released and, by means of another filtration through the cartridge, specifically adsorbed on the anion exchanger. Then the cartridge is washed with 0.8 M NaCl, 50 mM Tris-HCl buffe, pH 7.5, 1mM EDTA, and the DNA is eluted with about 1 ml of 1.2 M NaCl, 50 mM Tris-HCl buffer, pH 7.5, 1 mM EDTA.

Column 12, lines 63 to 69 [Example 3]:

"...and the phage pellet is dissolved in 20 µl of 10 mM Tris, 1 mM EDTA, pH 7.5. Another part by volume of extraction buffer (2% Triton X-100®, 7 M urea, 100 mM EDTA, pH 7.5) is added, and the mixture is heated at 50 °C for 15 minutes to release the single-stranded DNA."

Column 13, lines 14 to 24 [Example 4]:

The isolation of cellular DNA from sperm is carried out as follows:

One hundred µl of sperm are suspended in 1 ml of 500 mM NaCl, 10 mM EDTA 40 mM DTE, 10 mM Tris-HCl buffer, pH 7.5, 1% Triton, 4 M urea and 20 µg/ml of proteinase K and incubated at 37 °C for 2 hours. After centrifugation at about 5000 g for 5 minutes, the supernatant is passed through the separating gel in a cartridge. The flow velocity of the supernatant through the cartridge is about 1 ml/min.

Column 13, lines 64 to 68 [Example 7]:

The preparation of CMV (cytomegalovirus) DNA from urine is carried out as follows:

CMV viruses are lysed in situ upon addition of 4 M urea, 1% Triton, 500 mM NaCl, 50 mM Tris-HCl buffer, pH 7.5

It must be stated that all these citations clearly show that Henco uses urea only for the purpose of lysis (as was already pointed out in our facsimile letter dated October 8, 1999). There is no hint to use urea as a chaoetropic substance in an elution buffer or for the purpose to wash nucleic acid adsorbed on a matrix. On the contrary, Henco discloses that the addition of urea to the

loading buffer (i.e., before the adsorption/binding of the nucleic acid) has no effect the binding behavior of the long chain DNA.

This can clearly be seen from the description column 7, lines 8 to 11:

The addition of urea to the loading buffer does not affect the binding behavior of the long-chain DNA, while it optimizes the separation efficiency with respect to proteins.

As can be seen, the only advantageous effect of the addition of urea results in a better separation efficiency with regard to the proteins. However, this is of no help at all with regard to the solution of the problem underlying the presently claimed invention. Even if one would like to argue that the addition of urea to the loading buffer has positive effects with regard to the separation of nucleic acid from proteins during the following elution from the porous matrix and if - assuming arguendo - one would like to follow the line of argumentation of the Examiner that urea implicitly mans the use of any substance which destroys the order of water in which it is dissolved (i.e., a chaotrope) the advantageous effect of protein separation will only show relevant for unpurified lysates.

It must be noted that according to the presently claimed invention the mixture resulting from step b) and being the starting material of step c) is already purified and, thus, is free from proteins. Accordingly it cannot be seen that the teaching of Henco is of any help for the solution of the problem of the presently claimed invention because the use of urea or a chaotrope at such a late step is simply superfluous in the light of the disclosure of Little.

However, it cannot be seen that the use of urea as a lysis agent leads those skilled in the art to use chaotropic substances in a washing buffer, to wash nucleic acids which are bound on a matrix.

It should be born in mind that a skilled reader will study a document in a practical manner and in an afford to make sense of it. Thus, the skilled reader will discard any possible interpretations of the document which are illogical, impractical or fanciful. If the Henco citations is read in this manner, it will immediately be evident that Henco is no good point to start from if a skilled man is going to solve the problem underlying the presently claimed invention.

Little is concerned with a process for the purification of plasmid and other DNA, both single-stranded and double-stranded, by immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent and eluting the DNA with water or low salt buffer and a process for the immobilization of DNA onto diatomaceous earth in the presence of a chaotropic agent.

The invention is based on the finding that that diatomaceoius earth is useful for the purification of plasmid and other DNA by immobilizing the DNA onto the diatomaceous earth particles in the presence of a chaotropic agent, following by elution of the DNA with water or low salt buffer.

More particularly, the Little citation discloses a process for the purification of plasmid DNA comprising the following steps:

a) *immobilizing the DNA onto diatomaceous earth in the presence of a chaotropic agent;*

b) *washing the resulting diatomaceous earth-bound DNA with an alcohol-containing buffer;*

c) *removing the alcohol-containing buffer; and*

d) *eluting the DNA in a low salt buffer or in water.*

As already mentioned above, the invention is also directed generally to the immobilization of DNA onto diatomaceous earth which comprises contacting the DNA with the diatomaceous earth in the presence of a chaotropic agent.

According to Little a chaotropic substance is to be understood as a

> ... substance that enhances the partitioning of nonpolar molecules from a nonaqueous to an aqueous phase as a result of the disruptive effect that the substance has on the structure of water. Examples of chaotropic agents include sodium iodide, sodium perchlorate and sodium trichloroacetate.

[column 3, lines 36 to 43] (It should be noted again that sodium chloride forms not a part of the above mentioned chaotropic sodium salts).

It is no doubt true that the chaotropic substance is primarily used to bind the nucleic acid on the diatomaceous earth matrix.

However, Little discloses also to use a chaotropic binding buffer and a 50% ethanol buffer [column 4, lines 21 to 35]:

> An example of an alcohol-containing wash buffer comprises: 20.0 mM Tris-Cl pH 7.5, 20 mM EDTA, 0.4 M NaCl, and 50% v/v ethanol. This buffer will be abbreviated herein as "50% ethanol buffer" or "50% washing buffer.
>
> In order to lower the RNA and protein concentration in plasmid lysates, it is necessary to perform a sufficient number of washes using the chaotrope binding buffer and the 50% washing buffer. The amount of RNA and protein remaining is indirectly proportional to the number of volume washes performed on the diatomaceous earth pellet, membrane or column. Generally, about three washes of

> each buffer is sufficient to lower the RNA and protein concentrations to acceptable levels.

From the above it clearly can be seen that the washing steps using the chaotropic buffer on one hand and the washing buffer on the other hand are necessary to lower hte RNA and protein concentrations in plasmic lysates. However, similar to Henco, this step is necessary when using complex and not pre-purified lysates as starting material.

On the other hand it must, again, be noted that according to the presently claimed invention the mixture resulting from step b) and being the starting material for step c) is purified and, thus, is free from RNA and proteins.

Moreover, it should be noted that Little's intention with regard to the elution of the DNA was to optimize the yield of DNA to be recovered [column 4, lines 36 to 46]:

> The efficiency of release of immobilized DNA from the diatomaceous earth pellet, membrane or column will be proportional to the ratio of the volume of low-salt buffer or water added to the volume of the pellet, membrane or column. Thus, with a 5 μL diatomaceous earth pellet, for example, 5 μL of buffer or water (1 volume) will extract about 50% of the DNA. Likewise, 10 volumes of buffer or water added per volume of pellet will permit the recovery of >90% of the DNA. However, it should be kept in mind that the more buffer or water added, the more dilute the eluted DNA.

Accordingly, Little does not face at all the problem to isolate the DNA in a solution having a low salt concentrate.

Thus, appellants submit that the skilled artisan would not find Little a good place to start from when looking for teaching to solve the problem of the presently claimed invention. Furthermore, there was absolutely no motivation for a man skilled in the art to combine the teaching of both references - which both are concerned with DNA isolation from crude starting material - to reach the two step process of the presently claimed invention.

Even if - what is denied - one skilled in the art would have taken into account such a combination, it is clear - as discussed above - that he has to be inventive to find all the new parameters for such a two step isolation. The basic knowledge that urea does belong to the group of chaotropic substances would be of no or no essential help to arrive at a solution of the problem underlying the presently claimed invention.

The Examiner argues (on page 13, lines 14 to 22, of the final action):

> Therefore, Henco '426 use a combination of "urea, detergent and buffer" to effect lysis of CMV viruses (column 8, lines 60-62) is plainly an example wher etow of the three components of the lysis buffer are chaotripic (urea and detergent), a reality which hte lsysi buffer relies on the effect on cell wall lysis. Examiner concludes that applicant's argument is not convincing because there is no requirement that the prior art use any particular term to specify in the absence of the presence of specific term.

Appellant submits that this statement is incorrect. As was shown above, urea, when it was used in Henco, it was used in all cases in the lysis step. According to this disclosure it is no doubt true that the teaching of Henco clearly is, to use urea for the lysis (and not for the elution). The side effect of the better separation of the proteins from the nucleic acid is - as was shown of no relevance for

the use of some special chaotropic substances (as listed in claim 116) according to the presently claimed invention.

What the Examiner tries is to take the word "urea" completely out of context of the teaching of Henco, to search for a specific inherent feature (which is of no meaning for the use disclosed by Henco) and in the last step – after generalization (chaotropic substances) to find – according to his opinion – an appropriate other feature and use of such class of compounds (as washing buffers) and to build up a very artificial and highly impermissible hindsight reconstruction only based on this new feature.

As indicated above, each of claims 122-126 and 129-137 is independently patentable. No prior art disclosure is relied on to meet the additional claim limitations recited in each of these claims. Instead, according to the Examiner (final Office Action, page 4):

> The specific details of washing steps, the timing of steps, the specific selection of wash solution contents, and the physical characteristics of the anion exchange resin and mineral adsorbent (e.g., particle diameter, pore size, etc.) are deemed to be variables clearly within the purview of ordinary practitioner seeking to optimize the Henco and Little process steps for a specific situation. Therefore, the details of adsorbent choice or other standard performance parameters (e.g., the frequency of washes, the variation of ionic strength in wash solutions, etc.) are deemed to be the kind of variables properly within the realm of routine experimentation by an ordinary practitioner in the course of optimizing the process steps disclosed in the prior art of record. For these reasons, the instant claims, insofar as they are directed to routine changes in experimental details of the kind noted above, are deemed to lack an adequate basis for a finding of patentable distinction for any variation of the instant claimed process, as such variations are deemed to have been properly included within the scope of the noted prior art.

Reliance on *deeming* what is disclosed in the prior art and *optimizing* claim variables, instead "Reliance on *per se* rules of obviousness is legally incorrect and must cease." *In re Ochiai*, 37 USPQ2d 1127, 1129 (Fed. Cir. 1995). If the prior art fails to disclose a rationale for varying parameters to be result effective, it can not have been obvious to choose the claimed parameter. *In re Antonie*, 195 USPQ 6 (CCPA 1977). Obviousness cannot be based on speculation.

> The examiner should be aware that "deeming" does not discharge him from the burden of providing the requisite factual basis and establishing the requisite motivation to support the conclusion of obviousness. . . . The examiner's reference to unidentified phantom prior art techniques . . . falls short of the mark.

Ex parte Stern, 13 USPQ2d 1379, 1382 (BPA&I 1989).

> Whether the changes form the prior art are "minor", as . . . [patent challenger] argues, the changes must be evaluated in terms of the whole invention, including whether the prior art provides any teaching or suggestion to one of ordinary skill in the art to make the changes that would produce the . . . [claimed] method and device.

Northern Telecom, Inc. v. Datapoint Corporation, 15 USPQ2d 1321, 1324 (Fed. Cir. 1990). Differences between the claims and the prior art do not amount to "an obvious design choice," when the differences "achieve different purposes." *In re Gal*, 25 USPQ2d 1076, 1078 (Fed. Cir. 1992). Where the *optimization* of a claim variable was not recognized in the art as effecting the claimed result, the result is unobvious. *Antonie*, 195 USPQ at 8. That a difference with the prior art amounts to an alleged "optimal condition . . . is not a substitute for some teaching or suggestion supporting an obviousness rejection." *Rijckaert*, 28 USPQ2d at 1957. When obviousness of a claim limitation is grounded on its allegedly being "old and well known in the art . . . as a means of optimization

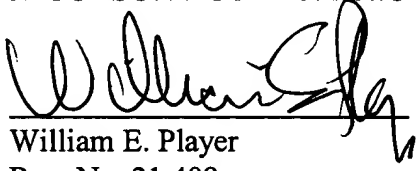
which is highly desirable," the "ground of rejection is simply inadequate on its face . . . because the cited references do not support each limitation of [the] claim." *In re Thrift*, 63 USPQ2d 2002, 2008 (Fed. Cir. 2002).

CONCLUSION

For the foregoing reasons, reversal of all appealed rejections of record is requested.

Respectfully submitted,

JACOBSON HOLMAN PLLC

By: 

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CLAIMS

Claims 1-119 (cancelled)

120. A process for the isolation and purification of nucleic acids from cells comprising, in two separation/purification stages, the steps of:

i) in a first separation/purification stage,

a) digesting the cells containing nucleic acids, removing cell debris and thereafter subjecting the nucleic acids to anion exchange against an anion exchanger in a first buffer solution, which has a low ionic strength,

b) desorbing the nucleic acids from the anion exchanger by applying a second buffer solution, which has a higher ionic strength than the first buffer solution, effecting purified nucleic acids in the second buffer solution; and

ii) in a second separation/purification stage,

c) adsorbing the separation/purified nucleic acids in the second buffer solution onto the surface of a mineral support material, optionally in the presence of lower alcohols, poly(ethylene glycol), or a mixture thereof, and

d) desorbing the nucleic acids from the mineral support material by applying an eluant, wherein the eluant is water or a third buffer solution, which has an ionic strength lower than the second buffer solution, effecting twice-purified nucleic acids.

121. The process according to claim 101, wherein the stages i) and ii) are carried out in immediate succession.

122. The process according to claim 120, further comprising the step of, prior to the digesting step, subjecting the cells to centrifugation or filtration in order to remove undissolved components.

123. The process according to claim 120 further comprising, between the steps a) and b), one or more washing steps by applying a fourth buffer solution, which has a low ionic strength, optionally increasing ionic strength per washing step.

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124. The process according to claim 120 further comprising, between the steps c) and d), one or more washing steps by applying a fifth buffer solution, which has an ionic strength higher than the first buffer solution.

125. The process according to claim 120 further comprising, between the steps c) and d), at least one washing step by applying an aqueous alcoholic solution.

126. The process according to claim 120 further comprising, between the steps c) and d), a washing step by applying a solution having an ionic strength corresponding to a 1.5 molar sodium perchlorate solution and a pH of 5.

127. The process according to claim 120, wherein the isolated and purified nucleic acid has from 10 nucleotides to 200,000 nucleotides.

128. The process according to claim 120, wherein the mineral support material is silica gel, glass, zeolite, aluminum oxide, titanium dioxide, zirconium dioxide, kaolin, or diatomacae.

129. The process according to claim 120, wherein the anion exchanger has a porous or non-porous matrix having a particle size of from 1 to 250 μm.

130 The process according to claim 120, wherein the anion exchanger has a porous or non-porous matrix having a particle size of from 10 to 30 μm.

131. The process according to claim 120, wherein the mineral support is silica gel, in suspension, having a particle size of from 1 to 250 μm.

132. The process according to claim 120, wherein the mineral support is silica gel, in suspension, having a particle size of from 1 to 5 μm.

133. The process according to claim 120, wherein the anion exchanger has a particle size of from 1 to 250 μm and a pore diameter of from 1 to 2,500 nm.

134. The process according to claim 120, wherein the anion exchanger has a particle size of from 10 to 100 μm and a pore diameter of from 1 to 2,500 nm.

135. The process according to claim 120, wherein the anion exchanger has a particle size of from 1 to 250 μm and a pore diameter of from 100 to 400 nm.

136. The process of claim 125, wherein the aqueous alcoholic solution includes from 1 to 7 M sodium perchlorate, from 1 to 7 M guanidine-HCl, from 1 to 5 M sodium chloride, from

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1 to 6 M sodium iodide, or 1 M sodium chloride in a 20% alcoholic solution wherein the alcoholic portion of the alcoholic solution is selected from the group consisting of ethanol, propanol, isopropanol, butanol, poly(ethylene glycol), and mixtures thereof.

137. The process of claim 120, wherein the eluant is a buffer solution that comprises water and Tris at a pH value of from 5 to 9.

138. The process of claim 120, whereby the nucleic acids are plasmid or genomic DNA.